REMARKS

In accordance with the present invention, there are provided methods for preventing injury caused by cooling of tissue and for reducing the toxicity of vitrification solutions by reducing the amount of cryoprotectant needed to vitrify. Inventive methods also facilitate the rapid introduction and washout of cryoprotectant fluids without toxicity or osmotic injury. Preserved tissues can suffer from three distinct types of injury: 1) injury due to ice formation, 2) injury due to cryoprotectant toxicity, and 3) injury due to cooling itself, in the absence of ice formation. Prior to the present invention, the prior art failed to address injury due solely to the cooling of the sample. The prior art only provided methods for the prevention of ice nucleation, which is not relevant to the present invention. In contrast, the present invention provides crucial protection to tissues in a manner which was not available according to the teachings of the prior art.

By the present communication, paragraph [0001] of the specification has been amended to update the priority claim as requested by the examiner. Additionally, paragraph [0044a] has been added. No new matter is added by these amendments. The subject matter of the new paragraph is fully supported by the claims as originally filed.

In addition, by the present communication, claims 19, 31, 32 and 34 have been amended to define Applicant's invention with greater particularity. No new matter is added by these amendments as the amended claim language is fully supported by the specification and original claims. Claim 33 has been cancelled. Claims 19-32 and 34-45 remain pending in this application, with claims 19-28, 31-32 and 34-37 under active prosecution. Claims 29, 30 and 38-45 have been withdrawn from consideration. A detailed listing of all claims that are, or were, in the application is presented herewith, beginning on page 2, along with an appropriate status identifier.

Rejection Under 35 USC § 112, First Paragraph

The rejection of claims 20-24, 31-34 and 36-37 under 35 USC § 112, first paragraph, as allegedly failing to comply with the written description requirement is respectfully traversed. As currently presented, the claims are fully supported by the specification.

With respect to claims 20-24, Applicant respectfully disagrees with the Examiner's assertion that the specification does not clearly describe tonicity in the ranges 1 to 4 times isotonic; 1.1 to 2.7 times isotonic; 1.1 to 2 times isotonic; 1.1 to 1.5 times isotonic; and 1.2 to 1.5 times isotonic. (See page 4, lines 13-16 of the Office Action). Contrary to the Examiner's assertion, the specification provides ample written description of tonicity ranging from 1.0 to 4.0.

For example, support for tonicity ranging from 1 to 2.7 is clearly provided in Figs. 4 and 5. Fig. 4 shows tonicity between 1.0 and 2.2, with clearly defined data points corresponding to actual solutions at 1.0, 1.2, 1.5, 1.6, 1.7, 2.0, 2.1 and 2.2. Fig. 5 shows tonicity between 1.4 and 2.7, with clearly defined data points corresponding to actual solutions at tonicities of 1.4, 1.5, 1.6, 1.7, 1.8, 2.1, 2.2 and 2.7. See also paragraph [0078], providing additional description of Fig. 5. In addition, Table 1 demonstrates solutions with tonicities of 1 and 2 times isotonic; Table 2 demonstrates solutions with tonicities of 1.0, 1.2 and 1.5 times isotonic; Table 3 demonstrates solutions with tonicities of 1.0, 1.5 and 1.7 times isotonic; and Table 4 demonstrates solutions having tonicities of 1.0, 1.12, 1.21, 1.46 and 1.68 times isotonic. With respect to tonicity up to 4.0 times greater than isotonic, support is provided in the specification at paragraph [0051], lines 8-9, where it states, "the slices were transferred from 40% w/v DMSO in 4X RPS-2..." Additional support is found at paragraph [0052], lines 5 and 12, where 4X RPS-2 is again described. Thus, the specification provides ample written description of tonicity ranging from 1.0 to 4.0, including specific examples wherein tonicity ranges from 1.0 to 2.7.

With respect to claim 31, Applicant respectfully disagrees with the Examiner's assertion that cryoprotective agents are not clearly described in the specification. (See page 4, lines 16-20

of the Office Action). Contrary to the Examiner's assertion, the present specification provides ample description of cryoprotectants. Moreover, cryoprotectant is a term of art which is well understood by those of skill in the cryopreservation art.

Cryoprotectant, as consistently used throughout Applicant's specification, refers to both a single cryoprotectant, as well as mixtures of different cryoprotectants. See paragraph [0043], lines 11-13. For example, at paragraphs [0036] and [0037], the specification describes the use of DMSO as the cryoprotectant. At paragraph [0047] to paragraph [0052], the specification describes DMSO or Veg (a mixture of DMSO, formamide, and ethylene glycol) as the cryoprotectant. At paragraph [0058], the specification describes V16 (see lines 3-7) as the cryoprotectant. At paragraph [0059], the specification describes V2X; i.e., a cryoprotectant solution consisting of 52% w/v Veg in a 2x RPS-2 vehicle solution (see paragraph [0059], lines 1-4).

With respect to the term "anti-nucleating polymers" (as referred to in claims 31 and 32), Applicant respectfully disagrees with the Examiner's assertion that the application lacks adequate written description. (See page 4, lines 16-20 of the Office Action). As acknowledged by the Examiner, this term is found in the original claims. The issue, however, has been rendered moot by the removal of the term from claims 31 and 32.

Applicant respectfully disagrees with the Examiner's assertion that polyvinyl pyrrolidone having a mean molecular mass of 5000 daltons (as referred to in claim 33) is not clearly described in the specification. (See page 4, lines 16-20 of the Office Action). As acknowledged by the Examiner, this term is found in the original claims; see also the footnote to Table 3, wherein PVP having a mean molecular weight of 5,000 is described. However, in view of the cancellation of claim 33, this issue has been rendered moot.

Applicant further disagrees with the Examiner's assertion that the phrase polyethylene glycol having a mean molecular mass of approximately 1000 daltons (as referred to in claim 34)

is not clearly described in the specification. (See page 4, lines 16-20 of the Office Action). As acknowledged by the Examiner, this term is found in the original claims; and is now incorporated into the specification as part of newly added paragraph [0044a]; see also paragraph [0047], which cites and incorporates by reference, U.S. Ser. No. 09/400,793, filed Sept. 21, 1999 (now issued as U.S. Pat. No. 6,395,467). Table 6, Ref. No. 27-3 of '467 describes a solution containing 4% PEG 1000 (i.e., polyethylene glycol with a mean molecular weight of 1000 daltons).

Applicant further disagrees with the Examiner's assertion that polyvinyl alcohol-polyvinyl acetate copolymer at a total concentration of 0.1 to 0.7 times isotonic (as referred to in claim 36) is not clearly described in the specification. (See page 4, lines 16-20 of the Office Action). As acknowledged by the Examiner, this term is found in the original claims; and is now incorporated into the specification as part of newly added paragraph [0044a].

Applicant further disagrees with the Examiner's assertion that the addition of acetol (as referred to in claim 37) is not clearly described in the specification. (See page 4, lines 16-20 of the Office Action). As acknowledged by the Examiner, this term is found in the original claims; and is now incorporated into the specification as part of newly added paragraph [0044a]; see also paragraphs [0081] and [0088], where the specification describes a solution which includes 7% acetol.

In view of the above remarks, reconsideration and withdrawal of the rejection under 35 USC § 112, first paragraph, are respectfully requested.

Rejection Under 35 USC § 112, Second Paragraph

The rejection of claims 19-28 and 31-37 under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicant regards as the invention, is respectfully traversed. As presently presented, claims 19-28 and 31-37 clearly claim the subject matter which Applicant regards as the invention and are not indefinite.

Specifically, Applicant respectfully disagrees with the Examiner's assertion that the term "living system" is not well defined in the specification, and therefore may read on a whole animal or human being. (See page 5, lines 5-14 of the Office Action). The term "living system" is a term of art in biology and requires no further definition. As used herein, the term refers to single cells, as well as collections of cells (including whole animals). Furthermore, as described in U.S. Patent No. 6,395,467 Patent (incorporated by reference at paragraph [0047]), cryopreservation fluids are useful with proteins, organelles, cell extracts, cells, tissues, blood vessels, organs, artificial or engineered cells, tissues, blood vessels, organs or organoids, organisms, or other biological systems. The Examiner has provided no evidence to suggest that the present invention cannot be used on a whole animal cooled to below 0°C. Thus, the metes and bounds of the present claims properly encompass everything from a cell to a whole animal. Therefore, the claim terminology is not indefinite.

Applicant further disagrees with the Examiner's assertion that the term "tonicity from X to Y times isotonic," as found in claims 20-24, is allegedly unclear. (See page 5, lines 14-18 of the Office Action). Specifically, the Examiner's suggestions that the above quoted phrase might refer to either "tonicity greater than the effective osmotic concentration" or to tonicity being "the same as the intracellular concentration" are both incorrect. It is respectfully submitted that the range of tonicity specified in claims 20-24 is well defined and understood. For example, the phrase "tonicity is from 1 to 4 times isotonic" merely refers to the effective osmotic concentration of the solution ranging from being equal to the isotonic concentration of the living system (when tonicity is 1 times isotonic), to an effective osmotic concentration of the solution being 4 times greater than the isotonic concentration of the living system (when tonicity is 4 times isotonic). The tonicity is equal to the ratio of the effective osmotic concentration (i.e., osmolality of the carrier solution plus any added polymer) to the isotonic carrier solution osmolality.

The meaning of the terms "X times isotonic" or "Y times isotonic," and therefore the meaning of the range "X times isotonic to Y times isotonic," is further exemplified by reference to the operational examples provided in the specification, such as the following:

Paragraph [0040], for example, describes a 4-5X solution as one that is "about 4 to 5 times more concentrated than normal, designated as 4-5X."

Paragraph [0050], for example, describes 2X RPS-2 as RPS-2 "concentrated by a factor of two."

Paragraph [0051], for example, describes 4X RPS-2 as RPS-2 "concentrated four fold."

Table 4 also describes in detail the relationship between tonicity and osmolality when both are changed by adding polymers to a carrier solution, providing several specific tonicities defined by several specific elevations in osmolality.

Thus, for the reasons given above, claims 19-28 and 31-37 are not indefinite. Accordingly, reconsideration and withdrawal of the rejection under 35 U.S.C. § 112, second paragraph, are respectfully requested.

Rejection in View U.S. Pat. No. 6,616,858 to Fahy

The rejection of claims 19 and 31-36 under 35 U.S.C. § 102(e), as allegedly being anticipated by U.S. Pat. No. 6,616,858 (hereinafter "the '858 Patent"), or in the alternative under 35 USC § 103(a) as allegedly being obvious in view of the '858 Patent, is respectfully traversed. The present invention, as defined, for example, by claim 19, distinguishes over the '858 Patent by providing a method for the reduction or elimination of cooling injury in a living system, comprising administering a preservation medium which includes a carrier solution and at least one cryoprotectant agent at a concentration to prevent freezing at a temperature below approximately 0°C, thereby reducing or eliminating cooling injury to the living system. The '858 Patent does not describe such a method.

In contrast, the '858 Patent is directed to methods for the prevention of ice nucleation or sample freezing. The prevention of ice nucleation is different from the reduction or prevention of cooling injury, as is required by the present claims. See paragraph [0006], lines 1-2, "thermal shock... is injury caused by rapid cooling *per se*," paragraph [0007], lines 1-3, "chilling injury... damage caused by exposure to low temperatures *per se*," and paragraph [0016], lines 5-8, distinguishing between cooling injury and "injury caused by ice formation, which, by the definitions herein, does not constitute cooling injury" (Emphasis added). This is further supported in the present application at paragraphs [0069] and [0070], where it states

> [0069] FIG. 3 also indicates indirectly that cooling injury is biochemical and is not an artifact of ice formation. . . The lack of association of injury with ice formation is evidence that the results are not an artifact of inadequate protection of the tissue from ice formation, but instead represent a real biological effect and demonstrate real attenuation thereof.
>
> [0070] Furthermore, since these conclusions pertain even to tissues that have been vitrified (the estimated glass transition temperature for this solution is about -123°C.), it is apparent from FIG. 3 that all injury caused by vitrification and rewarming is attributable to the combination of cooling injury and cryoprotectant toxicity, both of which are addressed by the present invention.

Thus, the present invention provides a method whereby living systems are protected from the effect of cooling per se at temperatures below 0°C, under conditions that would be damaging even if no ice formation occurred. Cooling injury is unrelated to ice formation, and the '858 Patent does not provide a method for reducing or eliminating cooling injury.

As noted previously, preserved tissues can suffer from three distinct types of injury: 1) injury due to ice formation, 2) injury due to cryoprotectant toxicity, and 3) injury due to cooling itself. The '858 Patent does not address injury due solely to the cooling of the sample. Instead, the '858 Patent provides methods for the prevention of ice nucleation, and damage caused therefrom. In contrast, the present invention is useful for the prevention of cooling injury which

may occur in kidney slices and whole organs cooled to temperatures above the freezing points of these systems, (see, for example, Examples 2, 3, and 6) wherein freezing (and therefore freezing injury) are not possible, yet injury due to the chilling is possible. This was neither taught nor suggested by the prior art.

Applicant respectfully disagrees with the Examiner's assertion that the '858 Patent provides the identical ingredients to the preservation fluid of the present application and provide the same function, which is minimal injury to the living system. (See page 8, lines 5-7 of the Office Action; emphasis added). As noted above, the present invention is directed to the reduction or prevention of injury due to cooling of the samples (e.g., thermal shock, chilling injury, or a combination of the two), a completely separate and distinct function than the prevention of ice formation. The '858 Patent does not describe or suggest such methods. In contrast, the '858 Patent is directed to the reduction or prevention of injury from cryoprotectant toxicity and ice formation.

Applicant further disagrees with the Examiner's assertion that the '858 Patent teaches methods for reducing or eliminating cooling injury, citing the abstract, Figs. 3-4, col. 1, lines 10-12, and col. 5, lines 1-6. (See page 7, lines 9-12 of the Office Action). There is no such discussion in any of these, or any other, sections of the '858 Patent teaching or suggesting the reduction or prevention of cooling injury to a living system. As noted above, the '858 Patent is directed to the reduction or prevention of ice formation and cryoprotectant toxicity, and therefore, is not in any way directed to the reduction or prevention of cooling injury, as required by the present claims.

Rejection of claims 19 and 31-36 over the '858 Patent is therefore improper. Accordingly, reconsideration and withdrawal of the rejections under 35 U.S.C. § 102(e), or in the alternative under 35 USC § 103(a) over the '858 Patent, are respectfully requested.

Rejection Over Fahy (U.S. Pat. No. 6,616,858) in view of Fahy (U.S. Pat. No. 6,395,467)

The rejection of claim 37 under 35 U.S.C. § 103(a) as allegedly being obvious over the '858 Patent, in view of U.S. Pat. No. 6,395,467 (hereinafter "the '467 Patent") is respectfully traversed. (See page 9, lines 9-19 of the Office Action). As acknowledged by the Examiner, the '858 Patent does not disclose the addition of acetol to the cryoprotectant solution. (See page 9, lines 19-21 of the Office Action).

Applicant respectfully disagrees with Examiner's assertion that it would be obvious for one of ordinary skill in the art to combine the cryopreservation solution of the '858 Patent with acetol simply because the '467 Patent discloses a solution which includes acetol. The present invention, as defined, by claim 37, distinguishes over the '858 and '467 Patents by providing a method for the reduction or elimination of cooling injury in a living system, comprising administering a preservation medium having a tonicity sufficiently hypertonic to minimize cooling injury, and at least one cryoprotectant agent at a concentration sufficient to prevent freezing at a temperature below approximately 0°C, wherein said cryoprotectant agent includes acetol and at least one of dimethyl sulfoxide, formamide and ethylene glycol, thereby reducing or eliminating cooling injury to the living system. Neither the '858 Patent, or the '467 Patent, taken alone or in combination, describe a method for reducing or eliminating cooling injury, much less the use of an acetol containing solution for reducing cooling injury.

The '858 and '467 Patents are both directed to cryoprotectant solution(s) of reduced toxicity for a reduction or prevention of ice formation at low temperatures. There is no teaching in either the '858 or '467 Patents of a solution wherein cooling injury (i.e., chilling injury and/or thermal shock) is reduced or prevented. Furthermore, acetol is but one additive in one example found in the '467 Patent. There is no specific discussion to suggest the use of acetol in a solution designed to minimize chilling injury or thermal shock. Thus, there is no suggestion or motivation to combine the teachings of the '858 and '467 Patents; rejection of claim 37 is

therefore improper. Accordingly, reconsideration and withdrawal of the rejection under 35 U.S.C. § 103(a) over the '858 Patent, in view of the '467 Patent are respectfully requested.

In view of the above amendments and remarks, it is respectfully submitted that the pending claims are in condition for allowance. In the event any issues remain in view of this communication, the Examiner is encouraged to contact the undersigned at the telephone number listed below so that a prompt disposition of this application can be achieved.

The Commissioner is hereby authorized to charge any additional fees which may be required regarding this application under 37 C.F.R. §§ 1.16-1.17, or credit any overpayment, to Deposit Account No. 50-0872. Should no proper payment be enclosed herewith, as by a check being in the wrong amount, unsigned, post-dated, otherwise improper or informal or even entirely missing, the Commissioner is authorized to charge the unpaid amount to Deposit Account No. 50-0872. If any extensions of time are needed for timely acceptance of papers submitted herewith, Applicants hereby petition for such extension under 37 C.F.R. §1.136 and authorizes payment of any such extensions fees to Deposit Account No. 50-0872.

Respectfully submitted,

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